Epidemiology and control of hazards in pork production chain – SAFEPORK
One health approach under a concept of farm to fork

genotypes corresponding to genotypes specific to the farms of the pigs and not to the type of production. Some of our results on antibiotic resistance are similar those obtained in other studies carrying on organic or antibiotic-free animal production. Thakur and Gebreyes (2005) obtained a lower resistance for TET and ERY for their C. coli in pig production without antibiotic. Moreover, multi-resistance was greater in conventional pig production. Less CIP-resistant Campylobacter jejuni strains were also obtained in antibiotic-free chicken products (Price et al., 2005), restricted use or absence of antibiotic has therefore an impact on antibiotic resistance of Campylobacter. No difference for the virulence was observed between organic and conventional strains. The high prevalence of virulence genes was already described in other works on C. coli (Wieczorek and Osek, 2013), although cblA gene was found in only 20% of the C. coli strains of Acik et al., (2013). Only 7% of our strains have the virB11 gene as described before (Acik et al., 2013, Wieczorek & Osek, 2013). This gene is involved in the invasion (Bacon et al. 2002) and we observed that our strains with this gene had a greater invasive capacity than other.

Conclusion
No impact of the type of production was observed on the genetic diversity and virulence of our Campylobacter strains. The lower level of antibiotic resistance was monitored in C. coli strains for organic pigs may be related to the restricted use of antibiotics in this production and / or colonization of organic pigs with susceptible environmental strains. However, although significantly different or not between the two productions, the percentage of strains with resistance in organic pigs remain in some cases relatively high. These pigs are therefore able to bring in the food chain bacteria resistant to antibiotics. The high prevalence of most virulence genes and the same pathogenicity capacity, whatever the type of production, suggest that these pig strains have the ability to infect humans.

Acknowledgements
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Residual contamination detection and serovar distribution of Listeria monocytogenes isolates in pork slaughterhouse and cutting facilities in province of Quebec.
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Introduction
L. monocytogenes (L. mono) is recognised as a zoonotic foodborne pathogen. Its control is focused on the “Ready-to Eat” food production level. Recently, Health Canada had reinforced its “Policy on L. mono in Ready-to-Eat Foods”, highlighting environmental surveillance and control of meat processing facilities as important risk reduction tools. The industry wants to improve its management of L. mono risk, taking into account previous steps of meat production. Nowadays, few information are available on the presence, distribution and types of strains in the environments concerning this pathogen in pork slaughterhouses and cutting facilities in Canada. Our objective was to detect and described residual L. mono contamination and analyse serovars distribution in different areas in the pork production continuum and between slaughterhouses and cutting facilities in province of Quebec during one year period. Such data are a pre-requisite to achieve the optimization of the management measures by the industries.

Materials and Methods
Sampling: Four main slaughterhouses were involved, representing 60% of the volume of meat produced annually in province of Quebec. A total of 16 exhaustive samplings had been carried out in four different seasons in one year. Each sampling represented a total of 156 samples, they were distributed to characterise the different steps of the slaughter/cutting process: lairage (n=53), slaughter (n=18), carcass dressing (n=23), refrigeration (n=8) and cutting (n=54). A total of 2,496 samples were analyzed in the current study. All samples were performed after sanitation procedures and analysed following a sensitive bacteriological method. All samplings was performed on 900 cm2 surfaces, beginning with brushing, then wiping of target surfaces with D/E neutralizing broth moistened swabs, (DIFCO BD, Sparks, MD). L. monocytogenes detection, from environmental samples: The isolation of L. mono was performed according to a routine procedure validated against the Health Canada MFHPB-30 method. Primary enrichment started with a 48 h incubation at 30°C in a total of 100 ml University of Vermont medium 1 broth (UVM -1, Lab M, Heywood, United Kingdom). For second selective enrichment, 100 µl from primary enrichment were transferred into 10 ml tubes containing Fraser broth (Lab M, United Kingdom) supplemented by Ferric Ammonium Citrate (Fraser, selective supplement, Lab M, United Kingdom) and were incubated for 48 h at 37°C. Both enrichments were streaked onto specific Listeria spp. medium (Biokar Diagnostics, Rue des Quarante Mines, France) for 24h at 37°C. Presumptive L. mono colonies grown on Compass medium appeared as bluish colonies surrounded by a distinctive opaque halo. Two isolates were selected by sample in order to confirm the hemolysis activity (type B+) on sheep blood agar. Finally, biochemical analyses were performed using carbohydrates (xylose, mannitol and rhamnose) use in broths (DIFCO, BD, Sparks, MD). Multiplex PCR and agglutination based serotyping: All isolates were subjected to a multiplex PCR assay method to confirm the genus and species by amplification of pirs and pfjA genes, and at the same time genoserogrouping the strains into five molecular serogroups by amplification of four target genes lmo0737, lmo1118, ORF2819, ORF2110. A second PCR assay was conducted in order to detect flaA gene presence as described by Kérouanton et al. in 2010. In addition, commercial O antisera (Denka, Seiken Co., Ltd, Tokyo, Japan) were used according to the manufacturer’s instructions to conclusive identification of strain the serotype.

Results
Mean residual detection after one year survey was 10% (240/2,496 samples analyzed). The prevalence of L. mono over time revealed that, whatever the season considered, the residual contamination detection
was stable with 11% (52/468), 12% (55/468) and 10% (89/936) of positive results respectively in spring, summer and winter time, during the one year study. In the other hand, variability on detection distribution of \textit{L. mono} among five different stages of production in four plants, was reported (See Graphic N°1). Chilling area has been proved to be the most susceptible to residual contamination, 18% (23 samples positives out of 128 samples analyzed), following with carcass dressing area, cutting area, lairage pens and slaughter with 14% (50/368); 18% (23/128); 10% (89/864); 8% (68/848) and 3% (10/288), respectively. Further analysis were carried out with the purpose to determine if residual contamination frequencies were different according to “in contact or not with carcass” for the sampled surfaces at slaughterhouse environments.

From a total of 240 positive samples distributed in the environments, 64% corresponded to “not in contact with carcass surfaces”, whereas 36% to susceptible to enter in contact with carcass or meat surfaces. Additionally, among all “not in contact with meat surfaces”, the floor represented the most frequently contaminated site: 79% (122/154). Others were more sporadically implicated: walls 12% (18/154), doors 3% (4/154) and ceilings 3% (4/154). In relation to “surfaces with carcass contact”, positive samples 73% (63/86) were seen from several sites of the entire cutting lines as conveyor lines, equipment, saws and cutting boards. The cutting line had so been considered the most representative place to sample. Analyses of detection in environmental samples on surfaces without contact with carcass revealed that chilling floor 31% (10/32) is more able to reflect residual contamination, followed by dressing carcass 21% (20/96) and cutting areas 16% (13/80). See Graphic N°2.

Moreover, detection on surfaces in contact with carcass at cutting line were in first place the saws (a carcass cutting tool), 19% (6/32), followed by conveyor for 14% (21/80). It should be noticed for conveyors that above and below sides shown the same detection rates, 13% (21/160) and 12% (19/160) respectively.

Analysis of \textit{L. mono} detection by slaughterhouses showed strong inter-plant differences. The highest rate was shown for the plant C 16% (96/624). Otherwise, plant D represented the lesser residual detection rate 5% (32/624). See Graphic N°4.

This high rate for plant C was due to higher prevalences on lairage pens, carcass dressing and chilling areas, 16% (33/212), 29% (27/92) and 31% (10/32), respectively. Conversely, residual detection at plant D in chilling area was 0%. See Graphic 5.

Moreover, detection on surfaces in contact with carcass at cutting line were in first place the saws (a carcass cutting tool), 19% (6/32), followed by conveyor for 14% (21/80). It should be noticed for conveyors that above and below sides shown the same detection rates, 13% (21/160) and 12% (19/160) respectively.

In the other hand, this study revealed a high diversity of serotypes among isolates. Mainly, \textit{L. mono} strains were of serotypes 1/2a (IIA), 1/2b (IIB), 1/2c (IIA) for 35% (83/240), 31% (74/240) and 17% (40/240), respectively. And lower contribution for 1/2c (IIC), 4c (IVA), 4b,4e (IVB), 1/2a (IIC), 3a (IIC), 3b (IIB) and 3a...
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Concerning plant C, one recurrent serotype (IIB, 1/2b) was detected on lairage pen area, on floor, over the four continuous visits. Furthermore, the same serotype was found on floor of slaughter area at two different occasions. At carcass dressing area, two different recurrent serotypes were isolated, the IIB, 1/2b (detected on bung dropper and veterinary inspection floors), and the IIA, 1/2c. This last was seen from dorsal splitter and veterinary inspection floors, both over two visits. At chilling area two recurrent serotypes were identified, (IIA, 1/2a and IIA, 1/2c), on floors at two visits. In addition, at cutting area, three different repetitive serotypes were found on surfaces with carcass contact (as cutting boards, IIA, 1/2c and on conveyor line (IIA, 1/2c and 1/2a)), all of them at two different visits. Finally, for plant D, only at lairage pen could be seen one recurrent serotype (IIA, 1/2a) on floors, over 3 continuous visits.

### Discussion

Our results, obtained after cleaning operations, show us that residual contamination was 10%. Similar rate were reported by Chasseinaux et al. in 2002, and Ortiz et al. in 2010. They observed low residual contamination in pork meat processing plants and slaughterhouses (2.5% and 9%, respectively). High prevalence of *L. mono* was seen by these authors on surfaces not in contact with carcass or meat, such as floor drains. They proposed that unsatisfactory sanitary procedures were involved, which enable the persistence of the so call resident “house strains” (8). In the present study, hilling areas showed the highest prevalence and residual *L. mono* contamination with recurrent serotypes could be found, but often different from those detected on previous or further steps of the production. Our results contrast with previous findings by Larivière-Gauthier, et al. in 2014 in Quebec where 40% of positive results were obtained in one plant. Nesbakken et al. in 1996 indicated that chilling and cutting areas significantly increased the contamination of pork meat, reporting (71-100%) of prevalence. The different serotypes we found at these two places indicated that the situation differ in our study. But impact on meat contamination was not assessed. With the multiplex PCR-based serotyping method we used, high presence of three predominant serotypes: 1/2a, 1/2b and 1/2c was seen. These results were in accordance with previous findings reported by Giovannacci et al., in 1999. Importance of serotype 1/2a is increasing in the epidemiology of listeriosis in France (6). Different situation appears in Quebec, Canada, were database from LSPQ (Laboratoire de Santé Publique du Québec) reported frequent highly pathogenic 4b strains in human surveillance cases. (5). Such 4b serotype isolates were sporadically detected in our study. Further typing is required before to be able to consider this in a public health perspective.

### Conclusions

*L. mono* is present and distributed in all areas of slaughtering and cutting process among four slaughterhouses over one-year study in Quebec. Each plant revealed different contamination level among 4 visits during one year period. We cannot suggest that chilling and cutting areas are a significant cause of meat contamination as amplified stage. Further analysis is needed to confirm the frequency of circulation of single strains among slaughterhouses.

### References

Concerning plant C, one recurrent serotype (IIb, 1/2b) was detected on lairage pens area, on floor, over the four continuous visits. Furthermore, the same serotype was found on floor of slaughter area at two different occasions. At carcass dressing area, two different recurrent serotypes were isolated, the IIB, 1/2b (detected on bung dropper and veterinary inspection floors), and the IIA, 1/2c. This last was seen from dorsal splitter and veterinary inspection floors, both over two visits. At chilling area two recurrent serotypes were identified, (IIA, 1/2a and IIA, 1/2c), on floors at two visits. In addition, at cutting area, three different repetitive serotypes were found on surfaces with carcass contact (as cutting boards, IIA, 1/2c) and on conveyor line (IIA, 1/2c and 1/2a), all of them at two different visits. Finally, for plant D, only at lairage pens could be seen one recurrent serotype (IIIA, 1/2a) on floors, over 3 continuous visits.

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References

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Toxoplasma prevalence in Dutch slaughter pigs in the period 2012-2014

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Abstract

Toxoplasma gondii has frequently been named as one of the most important foodborne pathogens, in terms of its impact on human health. EFSA advised to include serological testing of pigs on T. gondii infections and audits of pig farms on risk factors for T. gondii infection (EFSA, 2011). In order to generate knowledge about the epidemiology and prevalence of T. gondii infections in pig herds we studied the long term seroprevalence on farms, persistence of infection and variation in results between and within farms. Sera which were routinely taken in Dutch pig slaughterhouses in the Netherlands for the serological monitoring of Mycobacterium avium infections in pigs (Hiller 2013) were also tested for anti T. gondii antibodies. Results of 120,666 sera, collected from January 2012 until August 2014, showed an average of 2% serological prevalence in pigs. Pigs from organic farms had a prevalence of 3,6%. Farm prevalence was much higher, ranging from approximately 30% for conventional farms to 90% for organic farms. Pigs delivered to the slaughterhouse during winter months had a higher prevalence than pigs delivered during summer months. It could be concluded that serological monitoring can be very useful in detecting farms infected with T. gondii. A test cut off of 20PP was the most appropriate.

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Introduction

To control Toxoplasma gondii infections, intervention measures can be taken in the animal reservoir. Research showed that prevalence of T. gondii infections in pig was related to management on farms (Kijlstra, 2004). The number of pigs with antibodies against T. gondii in free-range farms was larger than on farms where pigs are kept indoors only. The risk for T. gondii in pigs has also been associated with the presence of cats, occurrence of rodents and the degree of cleaning and disinfection. A change of management aimed at reducing these risk factors could thus contribute to the reduction of T. gondii infections in pigs. The European Food Safety Authority has proposed epidemiological indicators for controlling T. gondii infections in pigs and safeguarding it in the pork meat chain (EFSA 2011). The instructions can be used by pig farms and slaughterhouses to prepare a package of measures depending on the risk for T. gondii infection. The measures advised by EFSA include serological testing of pigs on T. gondii infections and audits of pig farms on risk factors for T. gondii infection. However, the ideas of EFSA are abstract, not tested and not yet translated into working systems. Moreover, serological tests were developed and validated but not prepared for use in a system to control T. gondii infections. Until now, no long-term serological studies have been carried out in slaughter pigs to look for antibodies against T. gondii. To generate knowledge about the epidemiology and prevalence of T. gondii infections in pig herds we studied the long term seroprevalence on farms, persistence of infection and variation in results between and within herds.

Materials and methods

Serum samples which were routinely collected in 5 slaughterhouses in the Netherlands for the serological monitoring of Mycobacterium avium infections in pigs (Hiller 2013) were also tested for anti T. gondii antibodies. At every delivery (a group of pigs from the same farm, delivered on the same date to one slaughterhouse) of pigs, blood samples were collected randomly from pigs during bleeding. Per delivery, 1, 2 or 6 samples were collected (criteria for the number of pigs sampled were based on the M. avium monitoring system). For the